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Focal lesions area feature of chronic inflammatory demyelinating polyneuropathy (CIDP)

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Abstract In a study designed to identify the neuropathological features typical of chronic inflammatory demyelinating polyneuropathy (CIDP), we reviewed the sural nerve biopsy findings in 105 patients with this disorder. The patients' mean age at biopsy was 49 years. In 65% of patients the disease had a progressive and in 35% a relapsing-remitting course. In 47% of cases the disorder was idiopathic; the remainder had various concurrent conditions. All sural nerve biopsy specimens showed varying amounts of active demyelination associated with onion bulbs (48% of cases), endoneurial edema (55%) and inflammatory infiltrates (25%). The immunopathological hallmarks were T cell infiltration with macrophagic activation and up-regulation of major histocompatibility complex (MHC) class II expression, without B cell infiltration or immunoglobulin deposition on myelin sheaths. In 30% of cases some myelin sheaths showed C3d deposition. Analysis of proinflammatory cytokine expression invariably showed interleukin-1 in perivascular and endoneurial ramified cells and tumor necrosis factor-α prevalently in epineurial macrophages, whereas it detected interferon-γ only in samples with perivascular inflammatory cells. This immunological pattern suggests that the cellular components of immunity play the major role in CIDP. In 19% of cases the neuropathological changes had a focal distribution. This distinctive feature corresponded to more active demyelination, more frequent detection of inflammatory infiltrates and more prominent immunological activation, suggesting that focal involvement is a possible step in the course of the disease.

Key words Immune-mediated demyelination · Inflammation · Cytokines · Focal pathological changes

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Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a peripheral nervous system disease that extends over 2 months and may have a subacute chronic progressive or relapsing-remitting course [1, 3, 4, 6, 22]. CIDP may occur either without risk factors or concomitant disorders (idiopathic form; CIDP-I) or in association with monoclonal gammopathies of unknown significance (CIDP-MGUS) [5, 9] or other systemic disorders such as diabetes mellitus, thyrotoxicosis, neoplasias, infections, or demyelinating lesions of the central nervous system (CNS) (secondary form; CIDP-S) [1]. These varied presentations suggest that the CIDP syndrome has diverse causes but a common immunopathogenetic mechanism. The clinical and electrophysiological features of this disorder are sensorimotor neuropathy, elevated CSF protein content, and reduced nerve conduction velocities with conduction blocks [3, 6, 26]. Nerve biopsy specimens reveal pathological changes consisting predominantly of ongoing demyelination and remyelination, onion bulb formation, interstitial edema and inflammatory infiltrates in the spinal roots and peripheral nerve trunks [3, 12, 23, 25], sometimes having a patchy multifocal distribution [7, 15, 16, 30].

In this study we reviewed the pathological findings in a large series of sural nerve biopsy specimens from patients with CIDP. Our aim was to describe the histopathological changes in biopsy specimens and to determine their reliability for establishing the diagnosis and for distinguishing the pathogenetic mechanisms responsible for the three forms of CIDP.

Patients, materials and methods

We examined the clinical, laboratory, electrophysiological and nerve biopsy findings of 179 patients referred to our Center with a diagnosis of CIDP from January 1980 to December 1996. The clinical assessment did not include a disability scale. We selected only patients who satisfied the criteria proposed by the Ad Hoc Subcommittee of the American Academy of Neurology, 1991, for

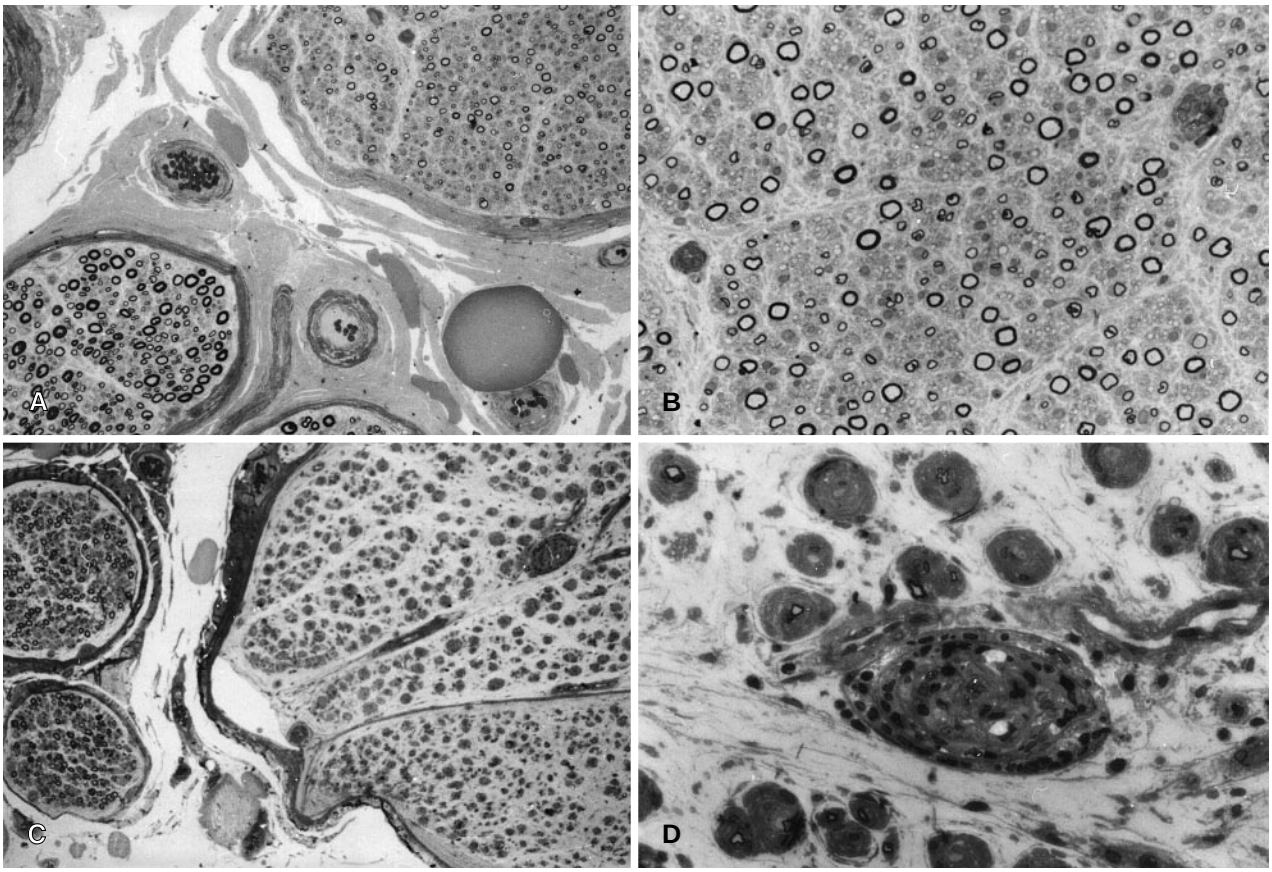

Fig. 1 A–D Transverse sections of sural nerve biopsy specimens from patients with chronic inflammatory demyelinating polyneuropathy. Note the focal distribution of the pathologic lesions in **A** and **C**. In **B** (higher magnification of **A**) nearly all the fibers in this fascicle are surrounded by thin myelin; in **D** note the endoneurial edema, demyelination, onion bulb formation and a perivascular inflammatory cell infiltrate. **A–D** Semithin sections, toluidine blue. **A** × 150, **B** × 325, **C** × 75, **D** × 300

a clinical, electrophysiological, laboratory and pathological diagnosis of CIDP.

Laboratory investigations

All patients underwent blood chemistry studies, immunological analyses and radiological investigations to exclude incidental metabolic disorders, nutritional deficiences, connective-tissue diseases, and malignancies. Serum immunoelectrophoresis was used to detect paraproteinemias. Most patients (88%) had lumbar puncture for CSF analysis.

Electrophysiological study

Patients underwent routine examination recordings of conduction velocities, temporal dispersion and distal latencies of the M wave, and F wave.

Nerve biopsy study

All patients underwent sural nerve biopsy under local anesthesia. The whole sural nerve was taken at ankle level. The specimen was processed according to our standard laboratory protocol, which includes frozen, paraffin and plastic-embedded samples for histology, immunohistochemistry, histometry and electron microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and observed on a Zeiss 109 Electron Microscope.

Immunocytochemistry

For immunocytochemical investigation a previously published protocol was applied [2]. A panel of monoclonal and polyclonal commercial antibodies was used to evaluate the immunophenotypical activation of T lymphocytes and their subsets (CD45Ro, CD4, and CD8; Dako, Glostrup, Denmark), B cells (CD20; Dako), macrophages (HAM 56; Enzo Biochem, New York, N.Y., and CD68; Dako) and HLA-DR class II expression (LN3; Clonab, Dreieich, Germany). Frozen sections of peripheral nerve specimens were examined by direct immunofluorescence using fluorescein-conjugated polyclonal antibodies that recognize human immunoglobulins, heavy (IgA, IgG, and IgM) light (kappa and lambda) chains, and the complement components C1q and C3d (Dako). The major proinflammatory cytokines [interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ); Genzyme, Cambridge, Mass.] were also assayed on frozen sections, using a standardized three-step avidin-biotin technique.

Results

Clinical features

According to the clinical, physiological and pathological criteria for the diagnosis of CIDP proposed by the Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force [6], 105 of the 179 patients were se-

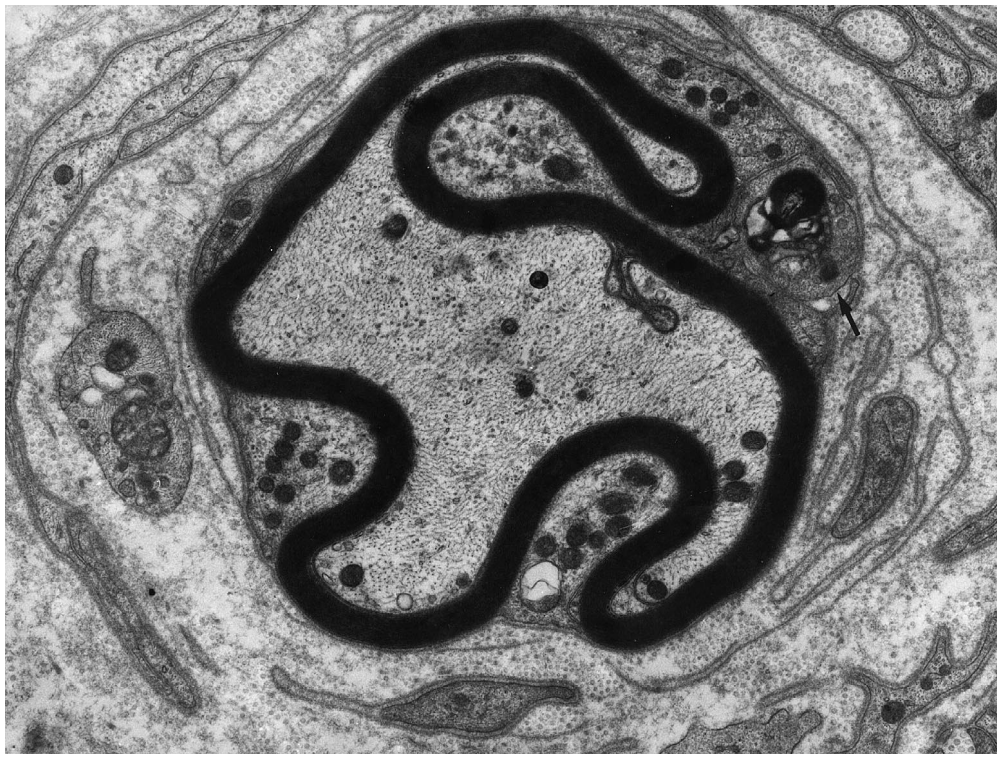

Fig. 2 Electron micrograph showing a remyelinating fiber surrounded by Schwann cell processes and empty basal membranes. Within the onion bulb layers there are some macrophagic processes and one of them, containing myelin debris, has penetrated under the basal lamina and is lying alongside a Schwann cell *(arrow)*. × 16 000

lected for this study. The other 74 patients failed to meet one or more diagnostic criteria. In particular, we excluded patients with MGUS who had anti-nerve antibodies [anti-myelin-associated glycoprotein (MAG), anti-sulfatide, or anti-GD1b] and patients whose sural nerve biopsy specimens showed no signs of demyelination.

Because CIDP may coexist with other diseases, our patients belonged to several categories: 46% had primary CIDP; 15.2% had concomitant monoclonal gammopathy (MGUS); 5.1% had CIDP secondary to neoplasias; and 33.7% had concurrent diabetes, systemic lupus erythematosus (SLE), HIV infection or CNS demyelinating disease. The clinical records showed that all these 105 patients had sensorimotor involvement with absent or reduced tendon reflexes, and rarely cranial nerve involvement. Ten patients had asymmetrical and multifocal distribution of sensorimotor involvement.

Most patients (74%) were male, a finding that suggests a predominant risk for men. The patients' mean age at the time of biopsy was 49 years. The wide age range (from 3 to 78 years) confirmed that the disease occurs at any age. In 35% of patients the disease had a relapsing-remitting or relapsing-progressive course, and in 65% a progressive course. The duration of the disease at the time of biopsy ranged from 6 months to 12 years.

Electrophysiological testing showed that all patients had reduced nerve-conduction velocities in the upper and lower limbs, increased distal latencies and prolonged F-wave latencies; 30% had nerve conduction blocks.

CSF examination disclosed increased protein content in 70% of patients, and normal values in 18% (12% did not undergo lumbar puncture).

Pathological findings

Light-microscopic examination of the plastic-embedded sural nerve sections from all patients showed thinly myelinated fibers and occasional naked axons. Other histopathological features varied in frequency. The most prominent were loss of myelinated fibers (severe in 30% of cases, mild in 55% and absent only in 15%), exuberant proliferation of Schwann cell processes to form onion bulb complexes (48% of biopsy specimens), enlargement of the subperineurial space and septa (55%), and perivascular infiltrates (25%). In 19% of all biopsy specimens, these pathological changes had a focal distribution and involved a variable number of fascicles or only part of a single fascicle (Fig. 1).

Teased-fiber analysis revealed active demyelination in all 105 nerve biopsies, although the amount varied widely: 25.5% of patients had segmental demyelination in 12–30% of internodes; 42% in 30–50% of internodes; 29% in 50–75% of internodes and 3.5% in more than 75% of internodes.

Neither the quality nor the quantity of these peripheral nerve changes differed in the idiopathic and secondary forms of CIDP.

Electron microscopy

The most common changes were demyelination and remyelination in various stages. Many myelinated fibers were surrounded by a very thin myelin sheath; all biopsy specimens contained naked axons with condensed fila-

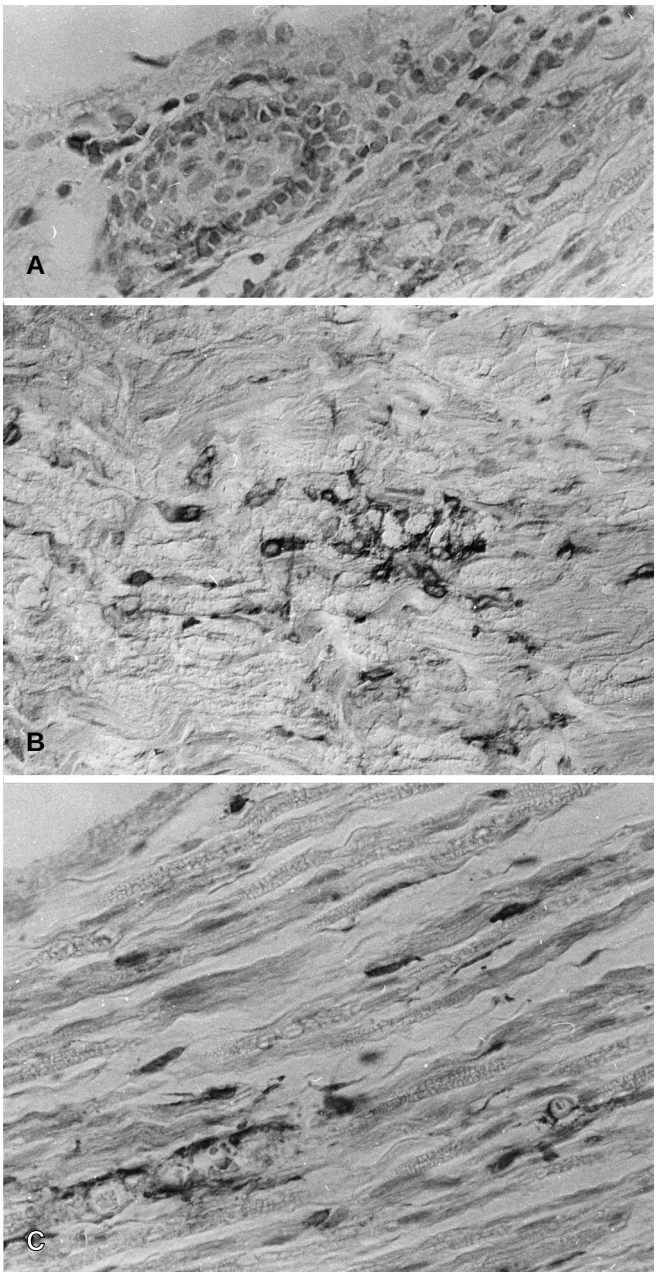

Fig. 3 A–C Immunohistochemical characterization of the inflammatory response in chronic inflammatory demyelinating neuropathy. **A** The perivascular inflammatory infiltrate predominantly contains CD45 Ro cells. Paraffin-embedded section. **B** $CD68^+$ cells are present in a perivascular infiltrate and scattered in the endoneurium. Paraffin-embedded section. **C** Immunostaining with LN3 shows increased HLA-DR class II expression on elongated ramified cells. Frozen section. **A** × 340, **B** × 370, **C** × 350

ments and tubules. Some fibers were surrounded by Schwann cell processes or by empty basal laminae, or both, arranged in concentric onion bulb complexes. In the vicinity of these fibers, occasional cells resembling macrophages had interrupted the basal lamina and invaded the cytoplasm of the Schwann cells (Fig. 2).

Immunocytochemistry

Immunophenotyping of inflammatory cells disclosed diffuse T cells both in the endoneurial and perineurial perivascular cuffs and scattered T cells in the endoneurium (Fig. 3 A), whereas B cells were found only in two cases, in the epineurial inflammatory infiltrate.

All sural nerve biopsy specimens contained $CD4^+$ and $CD8^+$ cells in almost equal percentages. A small number of $CD4^+$ cells had an elongated morphology resembling macrophages.

Macrophage-like $CD68^+$ cells were detected in all nerves arranged either diffusely in the nerve fascicles or among the T cells in the perivascular infiltrates (Fig. 3 B). The entity of cellular infiltration related strictly to the amount of active demyelination in the individual fascicles, and was more intense in those nerves with a focal distribution pattern.

Up-regulated major histocompatibility complex (MHC) class II expression was also found in elongated ramified cells suggesting resident macrophages (Fig. 3 C). None of the specimens contained immunoglobulin, kappa or lambda chain deposits on myelin sheaths; IgG were present within the plasma of blood vessels and in the perivascular spaces. In 30% of the biopsy samples, occasional C3d deposits were detected on some myelin sheaths.

IL-1 was expressed on T cells, epineurial macrophagic cells and endothelial cells and also in some elongated cells, probably resident macrophages or Schwann cells (Fig. 4 A, B). IFN-γ was almost exclusively expressed in the florid inflammatory infiltrates (Fig. 4 C), while TNF-α was expressed by inflammatory cells in the perivascular cuffs or also in the sparse inflammatory cells (Fig. 4 D).

Discussion

The diagnostic criteria for CIDP are usually based on clinical presentation, CSF analysis and electrophysiological findings, rather than on the histological features of nerve biopsy specimens [1, 6].

Autopsy studies have shown that inflammatory and demyelinative changes have a patchy, multifocal pattern of distribution, extending in most cases from the spinal roots to the distal peripheral nerves. However, in a chronic disease such as CIDP, the lesions can be found in different evolutionary stages depending on the duration of disease and the response to corticosteroid treatment. Therefore, the features of active inflammation and demyelination may be lacking in biopsy specimens, thus hindering diagnosis [1, 12, 15, 25, 26]. Our findings in this study, conducted on a large number of biopsy samples, confirm this drawback and attest that despite their variable incidence in the individual nerve biopsies specimens, the ongoing demyelination and remyelination changes are the pathological features required for the diagnosis of CIDP. Conversely, perivascular or diffuse infiltration of inflammatory cells correlate strictly with the temporal and spatial pattern of the disease.

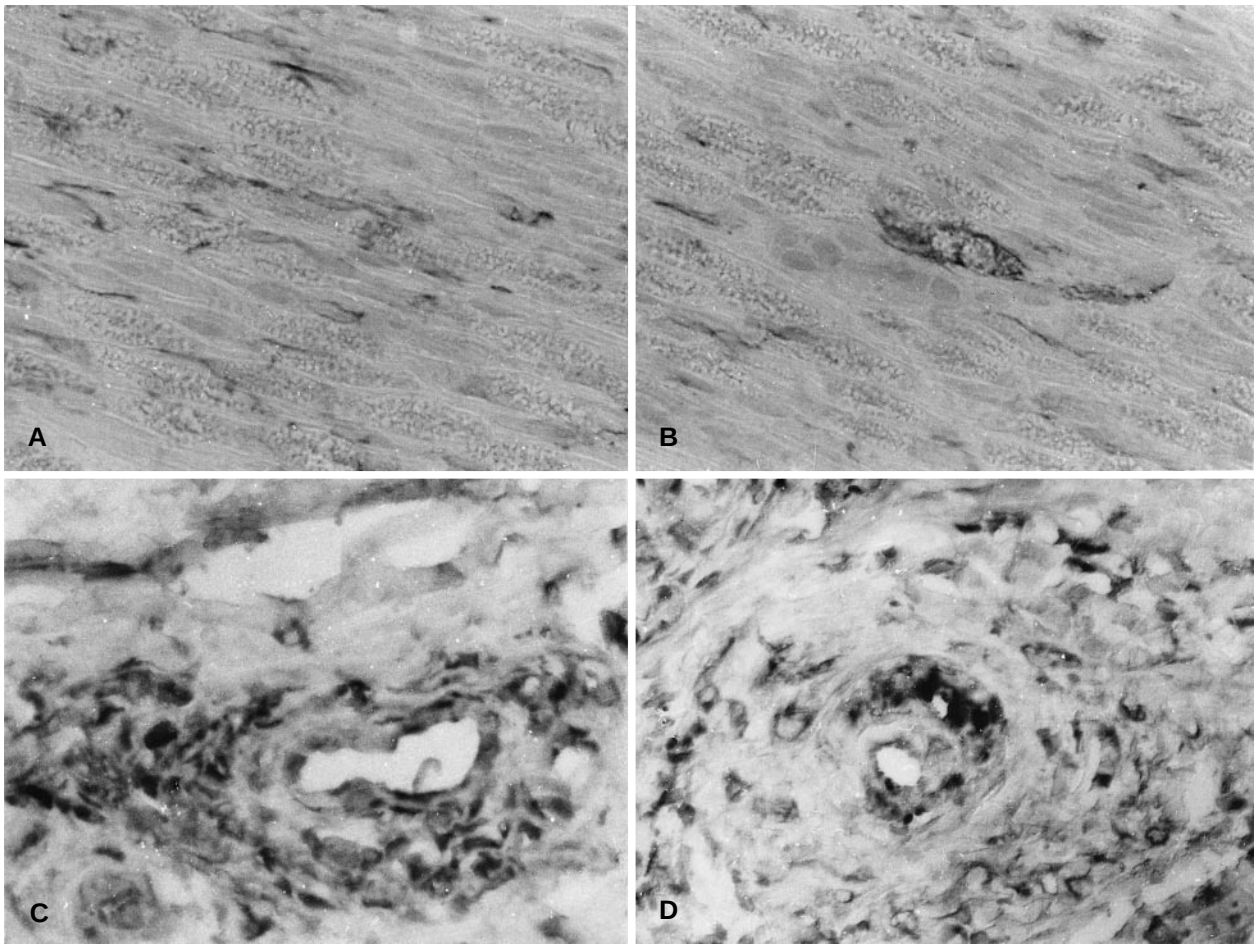

Fig. 4 A–D Cytokine expression in chronic inflammatory demyelinating neuropathy. **A, B** Diffuse interleukin-1 immunostaining in endoneurial cells: in **B** note immunodecoration of an elongated cell resembling a macrophage. **C** Interferon-γ expression is confined to perivascular inflammatory cells. **D** Tumor necrosis factor-α immunoreactivity is evident in sparse cells and in perivascular cuff inflammatory cells. **A–D** Frozen sections. **A** × 350, **B** × 380, **C** × 400, **D** × 500

Immunophenotyping identified the inflammatory cells either grouped in the perivascular cuffs or sparsely distributed in the endoneurium as T lymphocytes and activated macrophages, the latter showing up-regulation of MHC class II expression. Whether $CD8^+$ T cells have direct cytotoxic effects on Schwann cells or play a suppressor role remains unclear [21]. Observing that $CD8^+$ cells predominate over $CD4^+$ cells in some CIDP patients, Matsumuro et al. [15] suggested that $CD8^+$ T cells may function to maintain the demyelinating activity. Our observation that $CD8^+$ T cell subsets did not predominate over $CD4^+$ cells in the florid phases of inflammation argues against this interpretation.

Other investigators have described HLA-DR (MHC class II) expression in some elongated and ramified cells resembling Schwann cells [14, 17, 27]. In our cases, these cells were mostly $CD68^+$ cells, marker specific for identifying cells of mononuclear phagocytic origin and, thus, to be considered resident macrophages. This finding indicates that HLA-DR-expressing macrophages are the primary cells that present the antigen to lymphocytes [17, 21, 24]. Macrophages also intervene in the stripping and digestion of the myelin, a prominent feature in the acute phase of inflammation and demyelination. This stage of the process leads to Schwann cell proliferation and remyelination when numbers of newly synthesized cells exuberate and onion bulb processes form [23]. The constant and massive presence of cytokines in macrophages emphasizes the role of both cell types in the pathogenesis of CIDP [10, 11, 28, 29]. The constant immunodetection of IL-1 and TNF-α throughout the disease course, in cells resembling either macrophages or Schwann cells, strongly suggests a role for these cytokines in maintaining disease. The presence of IFN-γ in the very early phases of the process during the acute phase of the inflammatory demyelination, supports a role for this molecule in inducing but not in maintaining the disease, as demonstrated in the experimental model of experimental allergic neuritis. C3d detection on myelin fibers of 30% of our cases might suggest a possible role also for complement in initiating the immune-mediated myelin damage, through transient deposition of the terminal complement complex, opening membrane pores and activating influx of proteases. Together these data underline that in CIDP the cellular components of immunity play the major role. Conversely, the absence of B cells and immunoglobulin deposits argues

against a possible direct role for humoral immunity in inducing demyelination. The complement-mediated demyelination in patients with anti-MAG autoantibodies [18] and its experimental model by passive transfer [19] exhibit quite different features. Sural nerve biopsy specimens from these patients invariably show immunoglobulins and complement bound to the myelin sheaths and alterations in the periodicity of myelin.

An interesting feature in our CIDP series was that 22 of the 179 patients had distinctly focal disease. In biopsy specimens inflammation, macrophage-associated demyelination and even onion bulb formation were restricted to a single fascicle of a single nerve. Although we cannot exclude the possibility that focal cases are a pathological variant of the disorder, the clinical association with both symmetrical or asymmetrical neuropathy and the more active immunological pattern suggest that this multifocal expression of the disease is a frequent or even obligatory step in the evolution of the disorder. It would also explain the absence of lesions in nerve biopsy specimens from patients with a clinical and electrophysiological diagnosis of CIDP.

The possibility that demyelinative changes have a multifocal distribution also raises the question of the relationships between CIDP and the so-called Lewis-Sumner syndrome [13]. This rare entity, which has been described as "multifocal demyelinating neuropathy with persistent conduction blocks" is characterized either by asymmetrical focal impairment with a sporadic tendency toward generalization after years or by mild generalized sensory and motor neuropathy with superimposed multifocal nerve trunk lesions, predominant motor involvement and onset in the upper limbs [8]. Other reported features of the syndrome – considered an immune-mediated disorder on the basis of the responsiveness to corticosteroid treatment [20] – include preservation of some tendon reflexes, the presence of anti-GM1 antibodies, and a normal CSF protein content. In half of our cases the evident focal distribution correlated with features of progressive symmetric sensory and motor neuropathy, fitting the criteria of CIDP. These findings suggest that many patients classified under the Lewis-Sumner syndrome may well have a variant of CIDP rather than an individual clinicopathological entity.

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